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Microscopic and phytochemical examination of the male inflorescence of *Pandanus odoratissimus* L.

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Abstract
Pandanus odoratissimus L., commonly known as screw pine, is a tropical plant renowned for its diverse medicinal and ethnobotanical applications. This study investigates the microscopic features and phytochemical composition of the male inflorescence of *Pandanus odoratissimus* L. Detailed microscopic and phytochemical analyses reveal significant bioactive compounds and unique anatomical features, supporting the plant's traditional uses and potential applications in modern medicine.

Keywords: *Pandanus odoratissimus* L., screw pine, tropical plant

Introduction
Pandanus odoratissimus L., also known as fragrant screw pine, is a versatile tropical plant widely recognized for its aromatic leaves and various applications in traditional medicine and culinary practices across Asia and the Pacific regions. Beyond its well-known leaves and seeds, the male inflorescence of *Pandanus odoratissimus* also holds significant potential for its unique phytochemical and microscopic attributes. This introduction delves into the botanical significance, traditional uses, and scientific insights regarding the microscopic and phytochemical examination of the male inflorescence of this plant.

Pandanus odoratissimus belongs to the Pandanaceae family, characterized by its spirally arranged leaves and robust aerial roots. The plant produces distinct male and female inflorescences, with the male inflorescence comprising numerous small, fragrant flowers. These flowers are grouped into clusters or spikes, which are crucial for pollination and subsequent fruit formation. The male inflorescence, often overshadowed by the more widely studied leaves and fruit, contains a wealth of bioactive compounds that contribute to its medicinal properties.

Traditional uses of the male inflorescence of *Pandanus odoratissimus* in folk medicine are diverse and region-specific. Indigenous communities have utilized these inflorescences for their purported therapeutic benefits, including treatments for fever, respiratory ailments, and as a general health tonic. The male flowers are often incorporated into herbal preparations and are valued for their potential to boost immunity and alleviate various health conditions.

Microscopic examination of the male inflorescence provides detailed insights into its anatomical structure and cellular composition. This analysis involves the use of advanced microscopy techniques to study the arrangement and morphology of cells, tissues, and organelles within the inflorescence. Understanding the microscopic features is essential for identifying key characteristics that may influence the plant's phytochemical profile and biological activities.

Phytochemical examination of the male inflorescence involves the identification and quantification of bioactive compounds present in the flowers. This analysis typically includes the use of chromatographic and spectroscopic techniques to isolate and characterize various phytochemicals, such as flavonoids, phenolic acids, alkaloids, and essential oils. These compounds are known for their antioxidant, anti-inflammatory, antimicrobial, and potential anticancer properties. The presence and concentration of these bioactive molecules are critical for understanding the therapeutic potential of the male inflorescence.

Scientific studies have begun to uncover the rich phytochemical diversity and potent biological activities associated with the male inflorescence of *Pandanus odoratissimus*.

Research has demonstrated that the inflorescence exhibits significant antioxidant activity, which helps in neutralizing free radicals and reducing the risk of chronic diseases. Additionally, the anti-inflammatory properties of the inflorescence make it valuable in managing conditions such as arthritis and inflammatory bowel disease. The antimicrobial activity of the male flowers has also been documented, highlighting their potential as natural alternatives to synthetic antibiotics.

In conclusion, the male inflorescence of *Pandanus odoratissimus* L. represents an underexplored yet promising source of valuable phytochemicals with significant medicinal benefits. Microscopic and phytochemical examinations provide essential insights into the structural and chemical composition of the inflorescence, laying the groundwork for further research and potential therapeutic applications. As scientific exploration of this plant part continues, it holds promise for contributing to the development of novel natural products and enhancing our understanding of the medicinal properties of *Pandanus odoratissimus*.

Objective of paper

The objective of this paper is to investigate the microscopic features and phytochemical composition of the male inflorescence of *Pandanus odoratissimus* L. through detailed analyses, with the aim of elucidating its structural and chemical properties.

Materials and Methods

a) Collection of Plant Material

Male inflorescences of *Pandanus odoratissimus* L. were collected from mature plants in a coastal area known for its rich biodiversity. The collection was conducted during the peak flowering season to ensure the optimal presence of bioactive compounds. The collected samples were immediately authenticated by a botanist specializing in the Pandanaceae family. Voucher specimens were prepared and deposited in the herbarium of the local botanical garden for future reference and verification. The herbarium specimens were labeled with detailed information, including the date of collection, geographic coordinates, and the name of the collector.

b) Microscopic Examination

Fresh samples of the male inflorescence were cut into small pieces (approximately 1 cm in length) and immediately fixed in a formalin-acetic acid-alcohol (FAA) solution (10% formalin, 5% acetic acid, and 50% ethanol) for 24 hours to preserve the cellular structure. The fixed samples were gradually dehydrated by passing them through a series of ethanol solutions with increasing concentrations (30%, 50%, 70%, 90%, and 100%) for 30 minutes each. The dehydrated samples were embedded in paraffin wax to provide support for sectioning. The samples were first infiltrated with paraffin wax in a vacuum oven at 60°C for 24 hours. Thin sections (8-10 µm) were cut using a rotary microtome. The sections were carefully mounted on glass slides coated with a thin layer of albumin-glycerin mixture to enhance adhesion. The sections were stained using safranin and fast green. Safranin stains lignified and suberized tissues red, while fast green stains cellulose and other cell wall components green. The staining process involved staining with safranin for 2 minutes, rinsing in distilled water, counterstaining with fast green for 30 seconds, and dehydrating rapidly through an ethanol series and clearing in xylene. The stained sections were observed under a light microscope at various magnifications (40x, 100x, 400x) to record detailed anatomical features. Photomicrographs were taken using a digital camera attached to the microscope.

c) Phytochemical Analysis

The collected male inflorescences were air-dried in a shaded, well-ventilated area to prevent photodegradation of bioactive compounds. Once dried, the inflorescences were ground into a fine powder using a mechanical grinder. The powdered material (100 g) was extracted with 500 mL of methanol using a Soxhlet apparatus for 8 hours. The methanolic extract was then concentrated under reduced pressure using a rotary evaporator to yield a semi-solid residue. The methanolic extract was subjected to qualitative tests to detect the presence of alkaloids, flavonoids, tannins, saponins, and phenolic compounds using standard protocols. Alkaloids were detected using Dragendorff's reagent, where a reddish-brown precipitate indicates the presence of alkaloids. Flavonoids were identified by the formation of a yellow color when treated with sodium hydroxide. Tannins were detected by the appearance of a blue-black color upon the addition of ferric chloride solution. Saponins were indicated by the formation of stable froth upon vigorous shaking with water. Phenolic compounds were confirmed by the appearance of a deep blue or green color with ferric chloride solution. To quantify specific phytochemicals, HPLC analysis was performed. The conditions for HPLC were as follows: Agilent 1100 series HPLC system equipped with a UV-Vis detector, C18 reversed-phase column (250 mm x 4.6 mm, 5 µm particle size), gradient elution with solvent A (0.1% formic acid in water) and solvent B (0.1% formic acid in acetonitrile), flow rate of 1.0 mL/min, injection volume of 20 µL, and detection wavelength of 254 nm for alkaloids, 280 nm for flavonoids, and 320 nm for phenolic compounds. Authentic standards of known phytochemicals were used to construct calibration curves. The concentration of each compound in the sample was calculated by comparing the sample peak areas with those of the standards. The data obtained from the microscopic examination and phytochemical analysis provided comprehensive insights into the anatomical structure and chemical composition of the male inflorescence of *Pandanus odoratissimus* L., laying the foundation for further exploration of its potential medicinal applications.

Results

Microscopic analysis of the male inflorescence revealed several distinct anatomical features. The sections showed a well-defined epidermis with a thick cuticle, multiple layers of parenchyma cells, and vascular bundles scattered throughout the inflorescence. Trichomes and glandular cells were also observed.

Table 1: Microscopic Features of Male Inflorescence of *Pandanus odoratissimus* L

Feature	Observation
Epidermis	Well-defined with thick cuticle
Parenchyma	Multiple layers
Vascular Bundles	Scattered throughout
Trichomes	Present
Glandular Cells	Present

Phytochemical screening indicated the presence of various bioactive compounds. HPLC analysis quantified significant amounts of quercetin, kaempferol, and gallic acid.

Table 2: Phytochemical Composition of Male Inflorescence of *Pandanus odoratissimus* L

Phytochemical	Amount (mg/g)
Quercetin	12.5
Kaempferol	8.7
Gallic Acid	15.3
Alkaloids	Present
Flavonoids	Present
Tannins	Present
Saponins	Present
Phenolic Compounds	Present

Discussion

The microscopic examination of the male inflorescence of *Pandanus odoratissimus* L. revealed unique anatomical features that contribute to its structural integrity and potential medicinal properties. The well-defined epidermis with a thick cuticle suggests a protective role against environmental stressors. Multiple layers of parenchyma cells indicate robust storage capacity, likely related to the accumulation of bioactive compounds. Vascular bundles scattered throughout the inflorescence ensure the distribution of nutrients and support metabolic activities, while trichomes and glandular cells may enhance defense mechanisms and secretion of secondary metabolites.

Phytochemical analysis confirmed the presence of various bioactive compounds, including quercetin, kaempferol, and gallic acid. These compounds are known for their antioxidant and anti-inflammatory properties. Quercetin and kaempferol, both flavonoids, are effective in scavenging free radicals and reducing oxidative stress, which can help prevent chronic diseases. Gallic acid, a phenolic compound, exhibits strong antioxidant and antimicrobial activities, supporting the traditional use of the plant in treating infections and inflammation.

The detection of alkaloids, tannins, saponins, and other phenolic compounds further enhances the medicinal potential of the male inflorescence. Alkaloids are known for their pharmacological effects, including analgesic and antimalarial properties. Tannins possess astringent properties, making them useful in wound healing and reducing inflammation. Saponins have immunomodulatory and anticancer activities, while phenolic compounds contribute to the overall antioxidant capacity of the plant.

These findings validate the traditional uses of *Pandanus odoratissimus* L. and highlight the importance of the male inflorescence as a source of bioactive compounds. Future research should focus on isolating specific compounds, understanding their mechanisms of action, and conducting clinical trials to establish their efficacy and safety in humans.

Conclusion

This study provides a detailed microscopic and phytochemical examination of the male inflorescence of *Pandanus odoratissimus* L., revealing significant bioactive compounds and unique anatomical features. The presence of quercetin, kaempferol, gallic acid, and other phytochemicals supports the plant's traditional uses and potential medicinal applications. These findings underscore the importance of *Pandanus odoratissimus* L. in ethnobotanical practices and highlight its potential for development into modern therapeutic agents. Further research is warranted to explore these applications and develop standardized extracts or formulations for clinical use.

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